1976

Synthetic Ascorbigen as a Source of Vitamin C for Guinea-Pigs. II

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The effect of 26 mg of ascorbigen prepared from indole, formaldehyde, and ascorbic acid on guinea-pigs was reported in a preliminary communication[1]. Quantitative experiments with different doses of ascorbigen prepared from 3-hydroxymethylindole and ascorbic acid, as well as from indole, formaldehyde, and ascorbic acid, are reported in this paper. Paper chromatograms of both preparations were published in the preceding communication[2]. According to paper chromatography, the ascorbigen preparations contained < 1 μg of free ascorbic acid per mg.

In the animal experiments two different nutrient mixtures were used, neither of which contained any ascorbic acid according to paper chromatographic analysis.

Nutrient mixture I. A mixture of rolled oats and wheat embryos (4:1) *ad libitum* (the amount eaten in the beginning was about 30 g/day/animal). In addition 20 g of timothy hay (day/animal) which had been kept at 45°C for 3 days. The hay was of the same batch as that used in the preliminary experiment. If the lack of ascorbic acid was made good, the animals grew much better on this diet than on nutrient mixture II. Guinea-pigs of a pure albino strain, weighing 280 to 380 g, were fed on nutrient mixture I for 12 days, when symptoms of ascorbic acid deficiency became apparent. The animals were now divided into groups, 7 or 8 animals in each. 1 ml of saccharose solution (given *per os* by pipette daily) was included in the diet of the control group. Ascorbigen doses of 3, 7, 10, and 20 mg/day/animal in 1 ml of saccharose solution were given to the other groups. The experiment also comprised a group which received ascorbic acid. The solutions of ascorbigen and ascorbic acid were prepared immediately before pipetting.

Nutrient mixture II. The ascorbic acid-free diet of Harris and Olliver[3]: Ground whole oats 72 %, wheat bran 16 %, dried egg yolk 9 %, NaCl 0.5 %, $CaCO_3$ 1.0 %, $MgSO_4$ 0.5 %, cod-liver oil 1 %. The procedure described by Harris and Olliver was followed. Albino guinea-pigs, weighing 200 to 250 g, were at first fed on diet II *ad libitum* (the amount eaten in the beginning was about 30 g/day/animal). 15 g of cabbage/day/animal was given in addition, until the weight of the animals had increased to 300 g. Eleven days after this, the animals were given the test substances in the same manner as when nutrient mixture I was used. The groups fed on this diet comprised 6 or 7 animals.

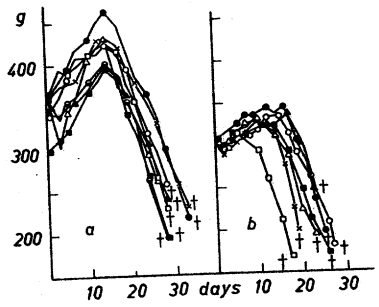

Fig. 1. Controls without ascorbic acid or ascorbigen. a. Diet I, b. diet II.

The weights of the animals during the test are shown in Figs. 1 to 6. Even3 to 5 mg of ascorbigen clearly retarded the development of scurvy. While the test animals on diet I died on the average 29 days (min. 26, max. 33 days) after the beginning of the experiment, and those on diet II after 23 days (min. 17, max. 27 days), the animals which were given 3 mg of ascorbigen (diet I) lived on the average 37 days (min. 28, max. 46 days) (Fig. 2a). 5 mg of ascorbigen lengthened the life of the animals on diet II from 23 to 39 days (min. 24, max. 56 days) (Fig. 5a). One animal in this group was still alive after 65 days, when it was killed. The odontoblast test revealed scurvy in this animal too, as could be concluded from the growth curve. Four animals died after 57 to 75 days in the group which had been given 7 mg of ascorbigen (diet I) (Fig. 2b). Three animals were still alive after 100 days. The animals which had been given 10 mg of ascorbigen (diet I) (Fig survived the test time of 100 days; c one animal died after 91 days in the gr in which ascorbigen prepared from ind formaldehyde, and ascorbic acid (Fig. was given. In the parallel group in wh 10 mg of ascorbigen prepared from 3 droxymethylindole and ascorbic acid (3a) was given, all the animals survived one attained a weight of 610 g, w corresponds to the growth of guinea which receive sufficient ascorbic Whether a different acidity of the ga juice should cause these variations wa investigated. The liberation of asc acid from ascorbigen is a comparat rapid reaction at the acidities of the g juice (Fig. 7).

The result thus indicates that animals can differ greatly in their a to utilize ascorbigen as a source of vit C. The animals which were given 10 m ascorbigen still suffered from lack of min C, with the exception of the s well-grown animal mentioned above. was also revealed by the odontoblas in the animals killed in each group 33 days.

The guinea-pigs fed on diet II and 15 mg of ascorbigen, grew on the a better than those which were given (of ascorbic acid (Figs. 5b and 6a) animals in the ascorbic acid grou (after 36 and 73 days), but none ascorbigen group.

The guinea-pigs grew as well on of ascorbigen as on 2 mg of ascorb (Fig. 4). According to the odontobla these animals had almost no further toms of deficiency after 33 days.

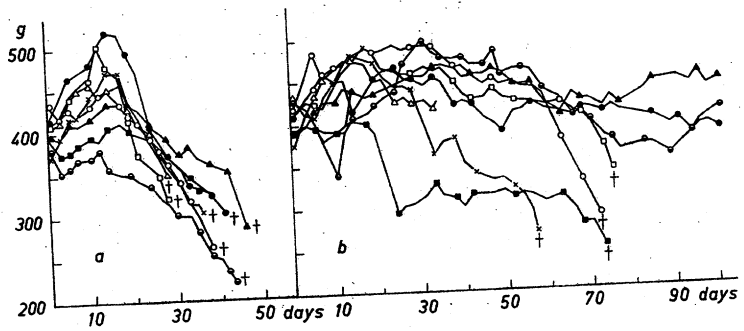

Fig. 2. Diet I. a. 3 mg of ascorbigen, b. 7 mg of ascorbigen. Both preparations sy from hydroxymethylindole.

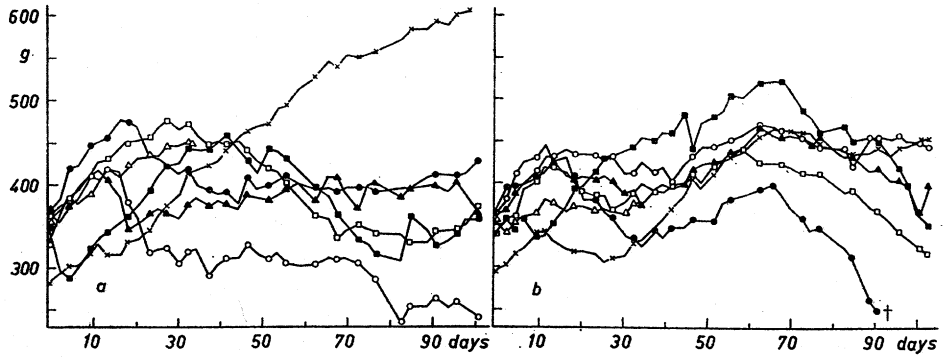

Fig. 3. Diet I. a. 10 mg of ascorbigen prepared from hydroxymethylindole, b. 10 mg of ascorbigen prepared from indole.

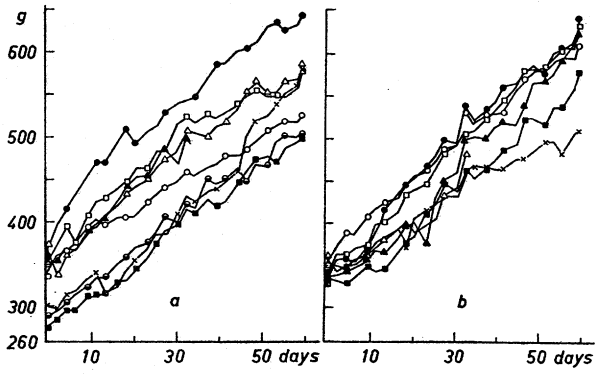

Fig. 4. Diet I. a. 20 mg of ascorbigen prepared from hydroxymethylindole, b. 2 mg of ascorbic acid.

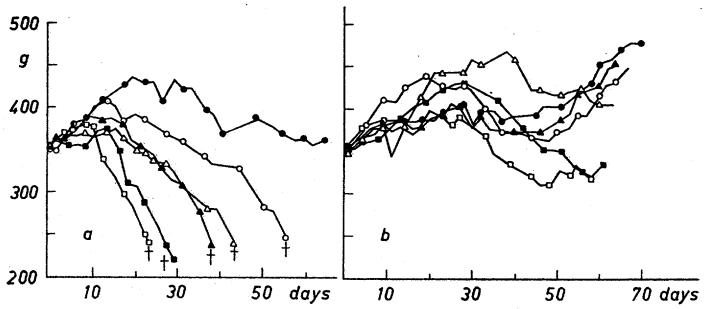

Fig. 5. Diet II. a. 5 mg of ascorbigen, b. 15 mg of ascorbigen. Both preparations synthesized from hydroxymethylindole.

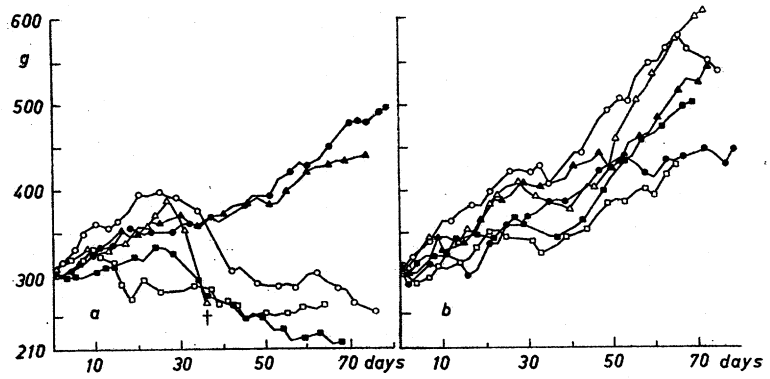

Fig. 6. Diet II. a. 0.6 mg of ascorbic acid, b. 3 mg of ascorbic acid.

The diet (I) of the guinea-pigs which were given hay was apparently more suitable than diet II. The difference was not due to vitamin C, however, since the animals which were fed on diet II (Fig. 6b), and which had also been given 3 mg of ascorbic acid, recovered slowly.

According to the results presented, guinea-pigs are on the average able to utilize about 10 to 15 % of the ascorbic acid bound in ascorbigen. The observation made in our first communication that 26 mg of ascorbigen has a curative effect on a scorbutic guinea-pig, was thus confirmed.

When using cabbage as a fresh vegetable, the formation of ascorbigen is of no practical importance. No decrease in ascorbic acid was found after normal chewing which lasted for 10—13 sec.

This research has been financed in part by a grant from the *United States Department of Agriculture, Agricultural Research Service.*

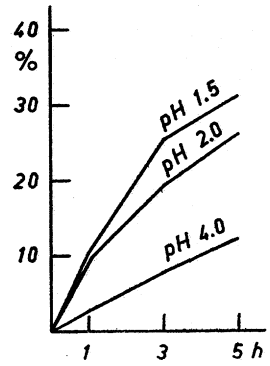

Fig. 7. The liberation of ascorbic acid from ascorbigen. The ascorbigen was hydrolyzed at 37°C in hydrochloric acid solutions, the pH values of which were 1.5, 2.0, and 4.0. The ascorbic acid was determined by titration both with dichlorophenolindophenol and dinitrophenylhydrazine according to the method of Roe. The results were confirmed by paper chromatography. The liberated ascorbic acid given as percentage of the bound ascorbic acid.

1. Kiesvaara, M. and Virtanen, A. I. *Acta Chem. Scand.* **16** (1962) 510.
2. Virtanen, A. I. and Kiesvaara, M. *Acta Chem. Scand.* **17** (1963) 848.
3. Harris, L. J. and Olliver, M. *Biochem. J.* **36** (1942) 155.

Received February 10, 1963.